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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/784,986	02/25/2004	Seiko Hirano	US-109	1388

38108 7590 03/31/2005

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EXAMINER	
GEBREYESUS, KAGNEW H	
ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 03/31/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/784,986	HIRANO ET AL.	
	Examiner	Art Unit	
	Kagnew H. Gebreyesus	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 May 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claim Objections

1. Claim 8 is objected to because of the following informalities: Claim 8 objected for the recitation "homologous recombination with the DNA occurs is disrupted". Applicants are required to amend this claim accordingly.

Claim Rejections - 35 USC § 101

1. 35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 1-5 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter.

In the absence of the hand of man, naturally occurring proteins and/or nucleic acids are considered non-statutory subject matter. *Diamond and Chakrabarty*, 206 USPQ 193 (1980). This rejection may be overcome by amending the claims to contain wording such as " An isolated and purified protein or nucleic acid " For examination purposes the claim is read as such.

Claim Rejections - 35 USC § 112

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claim 6 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The implication of the word "derived" is not clear. Does it mean that SEQ ID NO: 3 is isolated from a *Methylophilus* bacteria? Or is SEQ ID NO: 3 a homologue of a sequence found in a *Methylophilus* bacteria?

4. Claim 5 indefinite in the recitation of "under stringent conditions" as the specification does not define what conditions constitute "stringent". While page 7 line [0036] to [0037] attempts to describe a stringent condition, the description encompasses conditions such as hybridizable with only 70% DNA homology to SEQ ID NO: 3, which is beyond the scope of what is considered stringent in the art. Sequences with 70% homology may encode different proteins with different activities. As such it is unclear how homologous to the sequence of a gene encoding SEQ ID NO: 3, a sequence must be to be included within the scope of these claims. Claim 6 and 8 are rejected for the recitation of "from a chromosome " (claim 6) or "wherein a gene on a chromosome"(claim 8). Applicants need to amend claim 6 as: " from the genome" and claim 8 as: "wherein a polynucleotide on the genome".

Claim Rejections - 35 USC § 112

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-9 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while it may be enabling for an enzyme of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 3 transformed in a Methylophilus bacterium (claims 7 and 9) does not

reasonably provide enablement for any variant with lysine decarboxylase activity wherein the variant comprises substitution, deletion, insertion or addition of one or more amino acids of SEQ ID NO: 4 or any variant comprising an amino acid having 90% identity to SEQ ID NO: 4 (claims 1, 2). Likewise the specification while being enabling for a DNA of SEQ ID NO: 3 encoding the polypeptide of SEQ ID NO: 4, does not reasonably provide enablement for any polynucleotide variant that encodes an enzyme with lysine decarboxylase activity wherein the variant comprises substitution, deletion, insertion or addition of one or more amino acids of SEQ ID NO: 4 (claims 3, 5, 6) or any variant encoding an amino acid sequence having 90% identity to SEQ ID NO: 4 (claims 4). In addition while the specification may be enabling for a transformed Methylophilus bacterium in which the polynucleotide encoding lysine decarboxylase of SEQ ID NO: 3 is disrupted it is not enabled for a Methylophilus bacterium transformed with any such polynucleotide variant in which said variant polynucleotide is disrupted (claims 7-9) given that decarboxylation of L-lysine can be catalysed by more than one enzyme (e.g. the *cadA* and the *ldc* genes in *E.coli*). The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Claims 1-9 are so broad as to encompass any enzyme with lysine decarboxylase activity comprising modifications including substitution, deletion, insertion or addition to the polypeptide of SEQ ID NO: 4. Claim 3-6 are so broad as to encompass modifications including substitution, deletion, insertion or addition to the polynucleotide of SEQ ID NO: 3. And claims 7-9 are so broad that the modified Methylophilus may have a disrupted gene that is not encoded by a variant of SEQ ID NO: 3 (see alignment data on page 3 line [0015] of specification). The scope

of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polynucleotide and polypeptide sequences and strains of bacteria broadly encompassed by the claims. Since the nucleotide sequence encodes the amino acid sequence of a protein which in turn determines its structural and functional properties in a host cell, predictability of which nucleotide changes can be tolerated in the DNA and thus the protein's amino acid sequence and obtain the desired activity in a host cell requires a knowledge of and guidance with regard to which changes in sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure as determined by the DNA sequence relates to its function. However, in this case the disclosure is limited to the nucleotide and encoded amino acid sequence of only one lysine decarboxylase sequence and disruption of the same in a specific host cell.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a sequence where specific residue modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any molecule and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification to any DNA encoding a protein to be diminished or to be altered with each further and additional modification, e.g. multiple substitutions. In the case of the host cell the disrupted gene can a different L-lysine decarboxylase gene since the claims encompass any modifications including substitution, deletion, insertion or addition to the polypeptide of SEQ ID NO: 3.

The specification does not support the broad scope of the claims which encompass all modifications of any lysine decarboxylase with 90% identity to the polypeptide of SEQ ID NOS: 4 encoded by the DNA of SEQ ID NO: 3 because the specification does **not** establish: (A) regions of the DNA or protein structure which may be modified without effecting lysine decarboxylase activity or result in a different lysine decarboxylase gene that encodes a different protein all the while retaining lysine decarboxylase activity; (B) the general tolerance of the DNA encoding lysine decarboxylase to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including lysine decarboxylase with an enormous amount of amino acid modifications of the lysine decarboxylase of SEQ ID NOS: 4 encoded by SEQ ID NO: 3 which is disrupted in Methylophilus. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of polypeptides and polynucleotides having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claims 1-9 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

These claims are directed to a genus of variant DNA molecules (claims 3-6) encoding variant lysine decarboxylases (claims 1 and 2). The specification teaches the structure of only a single representative species of such DNA (SEQ ID NO: 3) and a single representative species of the protein of SEQ ID NO: 4 encoded by SEQ ID NO: 3.
However, the specification fails to describe any other representative species of the variant polynucleotides or variant polypeptides by any identifying characteristics or properties other than the function of the polynucleotides and the function of the encoded polypeptide with L-lysine decarboxylase activity. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Furthermore claims 7-9 are directed to a genus of mutant Methylophilus strains comprising said variant polynucleotide which are disrupted. The specification teaches the host Methylophilus comprising the disruption of a single representative L-lysine decarboxylase encoded by SEQ ID NO: 3. However, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of the disruption of an L-lysine decarboxylase. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the

claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim Rejections - 35 USC § 102

2. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

3. Claim 1 is drawn to a protein selected from the group consisting of a protein with an amino acid sequence of SEQ ID NO: 4 and variants of the same with one or more substitution, deletion, insertion or addition and has lysine decarboxylase activity.

4. Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by Sabo et. al. "Chemical properties of *E. coli* lysine decarboxylase including a segment of its pyridoxal 5'-phosphate binding site." Biochemistry 13:670-676 (1974). Sabo et al. disclose a sequence with 34.9% sequence identity to SEQ ID NO: 4. Given that claim 1 is drawn to any protein with lysine decarboxylase activity and encompasses any number of substitutions, deletion, insertion, or addition of one or several amino acid residues and has lysine decarboxylase activity, the enzyme disclosed by Sabo's reads on claim 1. Therefore the disclosure of Sabo et al. anticipates claim 1.

5. Claims 3, 5 and 6 are drawn to a DNA encoding a protein selected from the group consisting of a protein with an amino acid sequence of SEQ ID NO: 4 and variants of the same with one or more substitution, deletion, insertion or addition and has lysine decarboxylase activity.

6. Claims 3, 5 and 6 are rejected under 35 U.S.C. 102(b) as being anticipated by Kukuchi et al. "Novel Lysine Decarboxylase Gene and methods of producing L-lysine" (US 08/849,212). Kukuchi et al. disclose a DNA sequence showing 51.1% best local similarity to SEQ ID NO: 3 that encodes a lysine decarboxylase. Claims 3, 5 and 6 are anticipated because they encompass sequences with any number of substitutions, deletion, insertion, or addition of one or several nucleotide residues and encode a lysine decarboxylase. Therefore the DNA sequence encoding the protein having lysine decarboxylase activity disclosed by Kukuchi et al. anticipates claims 3, 5 and 6.

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

9. Claims 7 - 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kikuchi et. al. (US PAT 5,8276,98) and Gunji et al. (WO2000/61723/ US2003/0124687 A1). Applicant's inventions are drawn to a modified Methylophilus bacterium in which the lysine decarboxylase

activity has been either reduced or eliminated or wherein the polynucleotide sequence encoding said enzyme is disrupted such that its expression is suppressed resulting in reduction or elimination of the enzyme activity followed by the use of such a bacteria for the production of L-lysine from a culture media containing methanol as the major carbon source and collecting the L-lysine from the culture.

Kikuchi et al., teach a method of producing increased level of L-lysine by disrupting the L-lysine decarboxylase genes (cadA and ldc) in *E. coli* using a plurality of methods including substituting a normal polynucleotide in the genome of the bacterium by a modified polynucleotide or disrupting or polynucleotides using homologous recombination. Disruption of L-lysine decarboxylase polynucleotide in this bacterium leads to accumulation of L-lysine by virtue of a decreased rate of L-lysine degradation. The *E. coli* is grown in liquid media containing glucose as the main carbon source and the L-lysine is collected from the culture. However Kikuchi et. al. do not use Methylophilus bacteria.

Gunji et. al., teach the efficient production of L-lysine using a methanol assimilating bacterium (Methylophilus methylotrophus AS1) transformed with a mutant LysE gene derived from Coryebacterium for efficient recuperation of the L-lysine from the culture medium that contains a one-carbon compound (methanol) as the main carbon source.

Gunji et al. stress the benefit of utilizing Methylophilus bacteria in producing L-amino acids because methanol is available in large amounts and because of cost effectiveness. The difference between Kikuchi's inventions and the present invention is that the current applicants use a bacterium (Methylophilus) that utilizes a one carbon compound (methanol) as the major

carbon source as opposed to glucose (six carbons) utilized by *E. coli* as the major source of carbon.

It would have been obvious for a person of ordinary skill in the art to use Kikuchi's method that disrupts the L-lysine decarboxylase in E. coli to produce a higher levels of L-lysine by disrupting the same L-lysine decarboxylase in the Methylophilus strain of Gunji et. al. and ferment the culture in a medium that utilizes methanol as the major carbon source which is commercially more advantageous. One of ordinary skill in the art would have been motivated to do so because producing L-lysine from medium containing glucose as taught by Kikuchi et. al. would turn out to be more expensive. One of ordinary skill in the art would have a reasonable expectation of success since Kikuchi et.al. demonstrate the increased products of L-lysine by disrupting lysine decarboxylase and Gunji et. al. demonstrate the use of Methylophilus ~~both~~ in producing L-amino-acids from methanol. Therefore claims 7-9 would have been prima facie obvious to one of ordinary skill in the art.

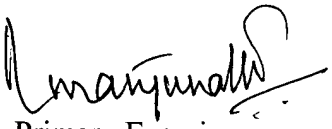
Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kagnew H. Gebreyesus whose telephone number is 571-272-2937. The examiner can normally be reached on 8:30 am- 5:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Achutamurthy ponnathapura can be reached on 571-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Kagnew Gebreyesus PhD.



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